



Optimal Viral Production

DANIEL COOMBS*

Theoretical Biology and Biophysics Group,
Theoretical Division, MS K710,
Los Alamos National Laboratory,
Los Alamos, NM 87545,
USA

E-mail: coombs@lanl.gov

MICHAEL A. GILCHRIST

Department of Biology,
University of New Mexico,
Albuquerque, NM 87131,
USA

E-mail: mike@unm.edu

JEROME PERCUS

Courant Institute of Mathematical Sciences,
251 Mercer Street,
New York, NY 10012,
USA

E-mail: percus@cims.nyu.edu

ALAN S. PERELSON

Theoretical Biology and Biophysics Group,
Theoretical Division, MS K710,
Los Alamos National Laboratory,
Los Alamos, NM 87545,
USA

E-mail: asp@lanl.gov

Viruses reproduce by multiplying within host cells. The reproductive fitness of a virus is proportional to the number of offspring it can produce during the lifetime of the cell it infects. If viral production rates are independent of cell death rate, then one expects natural selection will favor viruses that maximize their production rates. However, if increases in the viral production rate lead to an increase in the cell death rate, then the viral production rate that maximizes fitness may be less than the maximum. Here we pose the question of how fast should a virus replicate

* Author to whom correspondence should be addressed.

in order to maximize the number of progeny virions that it produces. We present a general mathematical framework for studying problems of this type, which may be adapted to many host–parasite systems, and use it to examine the optimal virus production scheduling problem from the perspective of the virus.

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1. INTRODUCTION

Viruses are infectious intracellular parasites that reproduce using machinery of a host cell. The viral particles produced (*virions*) transmit the viral genome to a new host cell, completing the cycle of infection. Assuming equal infectivity and survivability outside the cell, the reproductive fitness of a virus is proportional to the total number of progeny virions it produces. In a competitive situation of two viruses with different viral production rates but equal infectivity, survivability, and other features, the virus with the higher production rate will take over the population (Gilchrist *et al.*, submitted; Almgry *et al.*, 2002). The result that natural selection tends to favor viruses with the greatest basic reproductive ratio parallels that observed for other types of parasitic infections (Bremermann and Pickering, 1983; May and Anderson, 1983).

Once a virus has infected a cell, viral production may occur until the death of the cell. Infected cell death may occur by: (i) natural (background) mortality, (ii) cytopathic effects of viral replication and (iii) the host immune response to infection. Viral cytopathic effects and the ability of the immune response to detect and kill a virally infected cell will depend on the rate of viral replication. This leads to a situation in which the fitness of the virus may not be increased by higher replication: reproducing at too high a rate may lead to rapid cell death, in essence sabotaging potential reproduction from the infected cell in the future. If the loss of future production is greater than the short-term gain obtained from high replication rates, then natural selection will favor a decrease in viral production rates. Selection within the host will thus favor viruses that maximize their fitness within the constraints of this trade-off between present and future production. (Details are presented in Gilchrist *et al.*, submitted.)

One might thus envision different strategies for viruses that infect cells with different lifetimes. If a cell lives only a short time, such as an activated T lymphocyte, then natural selection may favor viruses that replicate as fast as possible, producing as many offspring as possible in the limited time the cell is alive. Viral cytopathic effects or immune responses may not appreciably shorten the lifespan of an already short-lived cell. In contrast, if a virus infects a longer-lived cell, such as a resting T lymphocyte or macrophage, then slow replication that does not trigger viral cytopathic or effective cell-mediated immune responses may yield more progeny than a rapid burst of replication that leads to cell death. Based on previous studies of parasite evolution (Sasaki and Iwasa, 1991), we also anticipate that the optimal strategy

may change over the lifespan of the infected cell; a fixed reproduction rate may be disadvantageous. In the case of HIV, viruses have been characterized as slow/low or rapid/high, where by slow/low one means a virus that grows slowly or generates low numbers of progeny (Fenyö *et al.*, 1988). Rapid/high is defined analogously. Thus, at least in the case of HIV, different replication strategies have been observed.

Here we address the following question: given a known (and fixed) relationship between the viral production rate, the time since infection, and the background mortality rate of the host cell, what viral production schedule in the absence of other constraints will optimize the total number of viral progeny? By production schedule we mean the rate of viral replication as a function of time since infection of a host cell. For example, one schedule is constant reproduction at a fixed rate P . Another is one in which the reproduction rate is initially 0 and then increases in a parameterized way to a maximum. In order to determine the optimal production schedule, we first restate the problem in a general mathematical framework. We then examine the effects of the cell mortality function on the optimal viral production schedule in different cases.

This paper is motivated in part by models of HIV dynamics *in vivo* in which it has generally been assumed that HIV is produced at a constant rate from cells once they are infected or after a brief delay (Perelson *et al.*, 1996; Mittler *et al.*, 1998; Perelson and Nelson, 1999; Nowak and May, 2001; Nelson and Perelson, 2002). However, from the biology of virus replication it is clear that a cell cannot instantly transition from no viral production to a constant rate of production. Thus, the question arises as to what function best describes the rate of virion production as a function of time since infection. In the case of HIV infection there are no measurements of the virion production schedule and there are conflicting estimates of the total number of virions produced over the lifespan of an infected cell (Haase *et al.*, 1996; Hockett *et al.*, 1999), a quantity called the burst size.

One approach to gaining insight and motivating experimental probes of this question is to calculate the optimal virus production schedule. A similar question was asked by Sasaki and Iwasa (1991), who in a pioneering paper posed this type of optimization problem in the context of pathogen production by an infected individual and its effect on transmission from one infected host to another. Almogly *et al.* (2002) asked a related question in the context of HIV infection. They focused on the idea that cells that produce more virus will be more visible to the immune system, and suggested that a strong immune response produces a selective advantage for latent viruses, whereas a deteriorating immune response provides a preferred environment for a higher replicating viruses. Their model was a quasi-species model in which each infected cell produced virus at a constant rate. However, they allowed different viral species to be produced at different rates and allowed infected cells to be killed at a rate dependent on their rate of viral production. We also consider the possibility that the rate at which cells are killed is dependent on their rate of viral production.

2. MATHEMATICAL MODEL

Let $P(t)$ be the virion production schedule, i.e., the production rate of virus particles per cell as a function of t , time since viral infection of the host cell. The mortality rate of the host cell, $\mu(P, t)$, is the rate at which host cells die as a function of the viral production rate, and time since infection. We assume that cell death is a first-order process with rate $\mu(P, t)$, so the probability of survival up to time t is exponential. We can thus define the survivorship to time t as

$$\sigma(t) = e^{-\int_0^t \mu(P(a), a) da}. \quad (1)$$

Although we call μ the death rate of an infected cell, in principle it includes any mechanism that halts virion production in the host cell. Many, but not all, of the known immune responses to viral infection involve killing the host cell. For example, replication of some viruses can be inhibited by the presence of interferons (Flint *et al.*, 2000; Janeway *et al.*, 2001).

We define the total number of virions produced by a cell since the time of infection at $t = 0$ by $N(t)$. The total number of virions produced by a cell from the time of infection, until cell death is called the *burst size* $\hat{N} = N(\infty)$. It is given by

$$\hat{N}[P] = \int_0^\infty P(t) e^{-\int_0^t \mu(P(a), a) da} dt. \quad (2)$$

Thus, the burst size is just the integral over time of the production rate (given that the cell is still alive) times the probability that the cell is still alive. Equation (2) suggests that if μ is an increasing function of P , there will be a trade-off between current production and future survivorship. As we shall show, whether this trade-off exists depends on the exact functional form of $\mu(P(t), t)$.

We examine the consequences of the hypothesis that natural selection within a host will favor the maximization of burst size. If such calculations give results in agreement with observation, it would provide some support for the optimization hypothesis and provide a possible explanation for the observation that resting CD4+ T cells have many fewer HIV RNA copies per cell than activated CD4+ T cells (Zhang *et al.*, 1999), consistent with different virion production rates in cells with different lifespans.

Therefore, our goal is to find the *optimal virion production schedule*, $P^*(t)$, which maximizes the burst size given a particular mortality function $\mu(P, t)$. Biologically, we must have $P^* \leq P_{\max}$, where P_{\max} is the maximum rate at which the host cell can produce virions. We will make the minor simplification that P_{\max} is constant over time, which need not be the case in the real system.

In constructing μ , we need an idea of how it will depend on $P(t)$ and t independently. The explicit time dependence of the function should include the background (uninfected cell) mortality function, as well as mortality effects of infection that are independent of the viral reproduction rate. For example, viruses such as HIV-1 that

integrate their genome into the host cell DNA can be transcriptionally active and yet not produce virions (Furtado *et al.*, 1999). If viral proteins are produced and presented on MHC class I molecules on the cell surface, cell-mediated immune responses should lead to cell death.

The dependence of μ on $P(t)$ will include the direct harmful effect that viral production will have on the host cell, due to utilization of cellular resources, e.g., depletion of nucleotide pools, cytotoxic effects of viral proteins, and the increased likelihood of a cell-mediated immune response when viral peptides are degraded and presented on the cell surface. These effects should be increasingly severe as viral production increases, and therefore we shall often think of the P -dependent parts of μ as increasing, concave-up functions. Further discussion of the form of μ , in a slightly different context, is found in Almogly *et al.* (2002).

If the background cell mortality rate depends on time explicitly then we should consider the age of the host cell at infection. The optimal viral production schedule would, in general, depend on that age, and the question would arise as to whether a virus would be able to determine the approximate age of its host cell. We shall assume that any time-dependent effects in the mortality function will begin at infection and therefore these issues are not examined here.

In the preceding discussion we have defined mortality μ to be a function of the production *rate* and time. A valid alternative model would be to consider μ as also a function of the intracellular viral *load* of the cell. Supposing that virions build up within the cell over time, this is equivalent to taking the cellular death rate to be a function of production to date. Doing this does not introduce any new difficulties. It is obvious that for $P(t) > 0$, $N'(t) > 0$. Mathematically, we can thus measure time by intracellular viral load. Biologically, we can interpret this as an increase in the effective age of the cell due to viral load. This reduction means that μ can be considered a function of $P(N)$ and N .

We begin by looking at the case in which infection has no effect on the death rate, and then build complexity by examining time dependence of the production rate, non-linear responses to production, full time dependence of μ and finally dependence on the viral load.

2.1. Cell death rate independent of viral replication. Suppose that the host cell is essentially unaffected by the infection, so that $\mu(P, t) = m(t) > 0$, where $m(t)$ is the background mortality rate, i.e., the death rate of an uninfected cell. In this case,

$$\hat{N}[P] = \int_0^{\infty} P(t) e^{-\int_0^t m(a) da} dt. \quad (3)$$

Because μ is not a function of P , there is no trade-off between present and future production. The virus should attempt to reproduce as quickly as possible; $P^*(t) = P_{\max}$ maximizes \hat{N} . This response is entirely independent of the age of the cell at infection.

2.2. Cell death rate linearly related to virus production. Suppose that viral replication leads to a simple linear mortality relationship $\mu(P, t) = \lambda P(t)$. The integral (2) can be performed explicitly, to find

$$\hat{N}[P] = \frac{1}{\lambda} (1 - e^{-\lambda \int_0^\infty P(a) da}). \quad (4)$$

The maximum value $\hat{N}[P] = 1/\lambda$ is achieved for a general set of functions $P(t)$ that have an unbounded integral on $(0, \infty)$. In a sense, all strategies the virus can take to maximize burst size are equivalent: reproduction now leads to an equivalent decrease in reproductive ability later.

A more realistic mortality function might be $\mu(P, t) = m + \lambda P(t)$, where λ is a constant and m represents a fixed background mortality rate. Because m is constant over time, the viral strategy will be independent of the age of the host cell at infection. In this case, the burst size integral is weighted by an exponential decrease over time

$$\hat{N}[P] = \int_0^\infty P(t) e^{-\lambda \int_0^t P(a) da} e^{-mt} dt. \quad (5)$$

If we constrain $P(t)$ to be constant, then $d\hat{N}/dP = m/(\lambda P + m)^2 > 0$. Therefore, the exponential weighting and the fact that future production is still discounted by current production together mean that it is best for the virus to reproduce as quickly as possible. The optimal fixed strategy is thus to reproduce at the maximum level for all time, $P^*(t) = P_{\max}$, and the optimal burst size turns out to be $\hat{N}[P^*] = P_{\max}/(m + \lambda P_{\max})$. For the case where $P(t)$ is a function of time, we integrate (5) by parts to get

$$\hat{N}[P] = \frac{1}{\lambda} \left(1 - m \int_0^\infty e^{-mt} \sigma(t) dt \right). \quad (6)$$

To maximize this, we must minimize the integral term. This is done by minimizing the survivorship function $\sigma(t)$, which is done by setting $P = P_{\max}$ for all time. In this case, therefore, the optimal dynamic production schedule is constant.

2.3. Non-linear relationship between cell death and viral production. We now allow μ to be a general function of P but one that does not depend on time except through $P(t)$. In particular, we have in mind that $\mu(P, t) = \mu(P)$ will be an increasing, concave up function of P , corresponding to an increasing rate of damage or toxicity to the cell as a function of viral production. A simple example is $\mu(P, t) = (\lambda/2)(P/P_{\max})^2 + m$, where m is a constant (examined in detail below). We present two approaches to finding the optimal production rate $P^*(t)$.

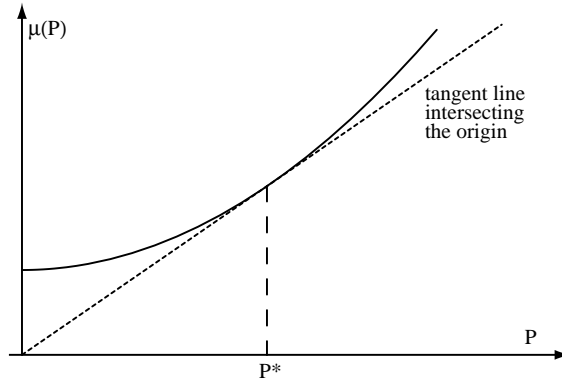


Figure 1. Graphical solution of equation (9). Solving (9) is equivalent to finding the value of P where the tangent line to $\mu(P)$ passes through the origin. As shown above, at P^* , $\mu(P^*) = \mu'(P^*)P^*$.

2.3.1. *Fixed production rate.* First, suppose that $P(t)$ is constant. The burst size is then given by

$$\hat{N}[P] = \int_0^\infty P e^{-\int_0^t \mu(P) da} dt = P \int_0^\infty e^{-\mu(P)t} dt = \frac{P}{\mu(P)}. \tag{7}$$

The optimal production rate, P^* , must satisfy

$$\left. \frac{d\hat{N}}{dP} \right|_{P=P^*} = 0 \quad \text{and} \quad \left. \frac{d^2\hat{N}}{dP^2} \right|_{P=P^*} < 0, \tag{8}$$

which in turn imply

$$P^* = \frac{\mu(P^*)}{\mu'(P^*)} \quad \text{and} \quad \mu''(P^*) > 0, \tag{9}$$

where $\mu'(P) = d\mu/dP$. In addition, for $P^* > 0$, we require $\mu'(P^*) > 0$. Equation (9) was first derived by Sasaki and Iwasa (1991) in a different context. The graphical solution of these equations for P^* is illustrated in Fig. 1. The existence of a solution, however, is not guaranteed by $\mu''(P) > 0$. To see this, consider the function $\mu(P) = P + (1 + P)^{-1}$ which is strictly concave up but yields no positive solution of (9). (Practically, in this case, the optimal production level will be $P = P_{\max}$.)

The conditions (8) apply only at the optimal production rate. $\mu(P)$ need not be concave up or even monotonically increasing over the interval $0 \leq P \leq P_{\max}$ for an optimal production rate to exist. A simple way to find the optimal production rate is as follows (see Fig. 1). Assume that $\mu(P) \geq 0$ and $\mu(0) > 0$. Lay a ruler on the P -axis. Rotate the ruler counterclockwise about the origin until it first

intersects the graph of $\mu(P)$ between 0 and P_{\max} . The coordinates of the point of intersection, $(P^*, \mu(P^*))$ satisfy $P^* = \mu(P^*)/\mu'(P^*)$ unless $P^* = P_{\max}$. Also, if $P^* \neq P_{\max}$ then it is easy to see that $\mu''(P^*) > 0$. If μ is concave down or linear, then $P^* = P_{\max}$. A proof that the production rate found in this way is optimal is given below in [Proposition 1](#).

2.3.2. *Dynamic production.* The dynamics of the probability that an infected cell is still alive at time t , $I(t)$, and the total number of virions produced so far, $N(t)$, are described by the first-order equations

$$\frac{dI}{dt} = -\mu(P(t))I(t) \quad (10)$$

and

$$\frac{dN}{dt} = P(t)I(t). \quad (11)$$

Note that $I(0) = 1$. Dividing (10) by (11), we have that

$$\frac{dI}{dN} = -\frac{\mu(P(N))}{P(N)} \quad (12)$$

so

$$I(N) = 1 - \int_0^N \frac{\mu(P(N'))}{P(N')} dN'. \quad (13)$$

Here we have written P as a function of N rather than t . This is legitimate provided $P > 0$ and so $N'(t) > 0$. The burst size \hat{N} is reached when $I = 0$, so

$$\int_0^{\hat{N}} \frac{\mu(P(N))}{P(N)} dN = 1. \quad (14)$$

To maximize \hat{N} , we must minimize the integrand for each value of N by choosing $P(N)$. Thus, it is sufficient to differentiate $\mu(P)/P$ with respect to P and set to zero. We then find after rearranging

$$P\mu'(P) - \mu(P) = 0, \quad (15)$$

found previously as [equation \(9\)](#). This result is independent of how P depends on N .

An alternative way of looking at the problem is to take the functional derivative of $\hat{N}[P]$ (details of this calculation are given in the [Appendix](#)), yielding

$$\frac{\delta \hat{N}}{\delta P} = P - \frac{\mu(P)}{\mu'(P)} + \frac{d}{dt} \left(\frac{1}{\mu'(P)} \right). \quad (16)$$

At extrema, the functional derivative is zero. Rearranging (16) under this condition gives a first-order differential equation to describe the evolution of extremal $P(t)$ through time,

$$\frac{dP}{dt} = \frac{\mu'(P)^2}{\mu''(P)} \left(P - \frac{\mu(P)}{\mu'(P)} \right). \quad (17)$$

We immediately see that the solution of (15) is a fixed point of this differential equation.

EXAMPLE 1. As an illustration, consider the case $\mu(P) = (\lambda/2)(P/P_{\max})^2 + m$. From equation (15), the optimal (fixed) production rate P^* is defined by $P^* = \mu(P^*)/\mu'(P^*) = ((\lambda/2)(P^*/P_{\max})^2 + m)/(\lambda P^*/P_{\max}^2)$, so $P^* = P_{\max}\sqrt{2m/\lambda}$, with burst size $\hat{N} = P^*/\mu(P^*) = P_{\max}/\sqrt{2m\lambda}$. As $m \rightarrow 0$, $P^* = P_{\max}\sqrt{2m/\lambda} \rightarrow 0$ and the burst size $\hat{N}[P^*] = P_{\max}/\sqrt{2m\lambda} \rightarrow \infty$, suggesting that for very long-lived cells with this mortality function the best viral strategy is slow production. This is consistent with the observation of hepatitis C virus, which infects long-lived hepatocytes, being produced slowly [cf. Boisvert *et al.* (2001)], as well as the observation of macrophage tropic viruses being slow/low (Fenyö *et al.*, 1988).

We now give an interesting alternative proof due to an anonymous referee of the fact that the best schedule is constant.

PROPOSITION 1. *For a mortality rate containing no explicit dependence on time that is a strictly positive function, $\mu(P) > 0$, the optimal production rate $P^*(t)$ satisfying $0 < P^*(t) < P_{\max}$ is static, i.e., time independent.*

Proof. We prove this proposition by finding a static production rate using the ‘rotating ruler’ argument described above, and then showing that it is optimal.

Define the set A by

$$A = \{a \geq 0 \mid \mu(P) \geq aP, \forall P : 0 \leq P \leq P_{\max}\}. \quad (18)$$

This is the set of all slopes $a \geq 0$ such that the line $y = aP$ lies below the graph of $y = \mu(P)$ for $0 \leq P \leq P_{\max}$. Define $\alpha = \sup A$ to be the slope of the steepest possible straight line through the origin lying below the graph of μ . The functions μ we are concerned with have background mortality, meaning $\mu(P) > 0$, so α exists and is positive. Moreover, there exists $0 < P^* \leq P_{\max}$ such that $\alpha P^* = \mu(P^*)$ and $\mu(P) \geq \alpha P$ for all $0 \leq P \leq P_{\max}$.

We compare this static strategy with an arbitrary strategy $P(t)$:

$$\hat{N}[P] = \int_0^\infty P(t) e^{-\int_0^t \mu(P(s)) ds} dt \leq \int_0^\infty P(t) e^{-\int_0^t \alpha P(s) ds} dt = 1/\alpha. \quad (19)$$

$$\hat{N}[P^*] = \int_0^\infty P^* e^{-\int_0^t \alpha P^* ds} dt = 1/\alpha. \quad (20)$$

Therefore the constant production rate P^* is optimal. This argument holds even when $P = P_{\max}$ (for example, when μ is a concave down function). \square

2.4. Explicit time dependence of the mortality rate with fixed production rate.

We now generalize μ by allowing it to depend on time explicitly, and focus on the case where P does not change over time: $\mu(P, t) = f(P) + m(t)$. The burst size is now given by

$$\hat{N}[P] = \int_0^\infty P e^{-\int_0^t m(a) da} e^{-f(P)t} dt \quad (21)$$

$$= P \int_0^\infty \sigma_1(t) e^{-f(P)t} dt \quad (22)$$

where σ_1 is the survivorship function for uninfected cells or the *natural survivorship*, i.e.,

$$\sigma_1(t) = e^{-\int_0^t m(a) da}. \quad (23)$$

Let the Laplace transform of the natural survivorship $\sigma_1(t)$ be S_1 , i.e.,

$$S_1(f(P)) = \int_0^\infty \sigma_1(t) e^{-f(P)t} dt, \quad (24)$$

so that

$$\hat{N}[P] = P S_1(f(P)). \quad (25)$$

To find the optimal production rate, we differentiate (25) with respect to P and set the result to zero:

$$0 = S_1(f(P)) + S_1'(f(P)) P f'(P). \quad (26)$$

This equation (with the condition $\hat{N}''[P] < 0$), which in general must be solved numerically, defines the optimal static value of P . We demonstrate with an example.

EXAMPLE 2. Let $\mu(P, t) = (\lambda/2)(P/P_{\max})^2 + \delta t + m$. Rescaling production $p = (P/P_{\max})$ and time $\tau = \lambda t$, consider maximizing the rescaled burst size $\hat{N}/(\lambda P_{\max})$. Setting $\epsilon = \delta/\lambda^2$ and $\phi = m/\lambda$, we have the rescaled mortality $\hat{\mu}(p, \tau) = (1/2)p^2 + \epsilon\tau + \phi$. This gives

$$\sigma_1(\tau) = e^{-(\epsilon/2)\tau^2 - \phi\tau} \quad (27)$$

and

$$S_1(x) = e^{(\phi+x)^2/(2\epsilon)} \sqrt{\frac{\pi}{2\epsilon}} \operatorname{erfc} \left(\frac{\phi+x}{\sqrt{2\epsilon}} \right). \quad (28)$$

The solution to (26) in this case can be found graphically for particular values of ϵ and ϕ . Figure 2 shows plots of (dimensionless) burst size as a function of p for varying ϵ , with $\phi = 0.1$. If background mortality is independent of time since infection, $\epsilon = 0$. Figure 2 clearly shows that in such a situation the virion production rate should be significantly less than P_{\max} .

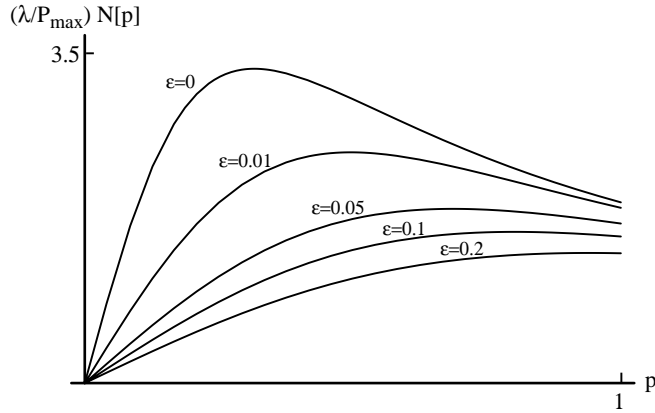


Figure 2. (Example 2) Using the dimensionless mortality function $\hat{\mu}(p, \tau) = \frac{1}{2}p^2 + \epsilon\tau + \phi$ for static p , dimensionless burst size is plotted against p , when $\phi = 0.1$ and $\epsilon = 0, 0.01, 0.05, 0.1, 0.2$. In each case, the maximum burst size occurs at the solution of (26).

It is now natural to ask whether in a situation with time-varying mortality a time-varying production schedule can be superior to the time-independent case shown here. In Appendix B, we show that, at least in some simple (but biologically natural) cases, time-varying production is advantageous.

2.5. Cell death rate dependent on cellular viral load. We now consider the case where the cell mortality depends on $P(t)$ and $N(t)$. We can choose to measure the effective age of the cell in physiological terms, as measured by the level of virus within the cell: if $N(t)$ is strictly increasing, we may write t as a function of N , and thus P as function of N .

Suppose first that $\mu(P, N) = \mu(N)$, a strictly positive function of only N . We must choose the production function $P(N)$ so that the burst size \hat{N} is maximized. The argument given in equations (10)–(14) applies here, so we again have the equality of equation (15),

$$\int_0^{\hat{N}} \frac{\mu(N)}{P(N)} dN = 1. \tag{29}$$

In order that the upper limit of integration, \hat{N} , is maximized in this equality, the integrand must be minimized. To this end, $P(N)$ must be made as big as possible. Therefore, the optimal production schedule in this case is a constant, $P = P_{\max}$ (as in Section 2.1).

If we now allow μ to be a positive function of P as well as N , with $\partial\mu/\partial P > 0$ and $\partial^2\mu/\partial P^2 > a > 0$, then the integrand in (29) becomes infinite as $P \rightarrow 0$ and as $P \rightarrow \infty$. An optimal value of P on the interval $0 < P < P_{\max}$ may therefore exist.

3. CONCLUSIONS

In order for viruses to propagate themselves, they must infect cells, and use the existing cellular machinery to produce new progeny virions. The number of progeny virions produced is thus influenced by a number of factors: the host cell resources available, the immune response that may kill an infected cell and limit its viral production, and factors intrinsic to the virus that control how rapidly it replicates. While natural selection within a host generally favors viruses which maximize the number of progeny produced over the lifespan of the infected cell, i.e. burst size (Gilchrist *et al.*, submitted) if viral production and cell survivorship are linked then replicating at the maximal rate may not be the optimal strategy for a virus. To determine the best reproductive strategy we formulated an optimization problem, in which the objective function to be maximized was the total number of progeny produced over the life of the infected cell, and the variable assumed to be under viral control was the rate of viral production as a function of time since infection. We then showed that the optimal viral production schedule depends on the mortality rate of the host cell. Since many viruses are cytopathic, i.e., they kill their host cell, we have allowed the infected cell death rate to be a function both of the time since cell infection, number of progeny virions produced and of the viral production rate. We showed that if the cell death rate was an increasing function of the viral production rate (independent of time) with second derivative bounded away from zero then there would be an optimal production rate that was lower than the maximum viral production rate possible. Further, we showed that even if the production rate was allowed to vary with time during infection, the optimal production schedule was to produce viruses at a constant rate.

We then examined the situation where the cell mortality rate explicitly increases over time. Grossman *et al.* (1999) suggested this possibility in HIV infection, although we know of no data that supports this hypothesis. Nevertheless, we find an interesting result. When mortality increases with time of infection, the optimal viral production rate is not constant, but increases gradually over time. This behavior reflects the decreasing value to the virus of future survival of the cell.

Finally, we considered the case where cell mortality is time-independent but depends on the amount of viral production to date. Biologically, this might correspond to the situation where viral proteins build up inside the cell and are responsible for its death. In this case, we found that the optimal strategy is for the virus to reproduce as quickly as possible. However, if the mortality also depends on the virion production rate, the optimal production rate may be lower than the maximum.

Our model was necessarily a simplification, but many refinements and further developments to add biological realism fit naturally into our time-structured framework. For example, our model placed no constraint on how rapidly viral production

could change. It is clear that cells cannot produce virus at a constant rate from the instant they are infected. Once a virion enters a cell there are many biological steps that must occur before new progeny virions can be produced. In the case of HIV, the virus has to enter the cell, the proteins that surround the viral RNA need to dissociate from the RNA (a process called uncoating), the RNA has to be reverse transcribed into DNA and the DNA needs to be transported into the nucleus and then integrated into the host cell's DNA. After that, the viral DNA needs to be transcribed into RNA, with some RNAs being multiply spliced and acting as messenger RNAs encoding viral proteins. In addition, full length transcripts are also made and are packaged into newly forming viral particles, that then bud from the infected cell surface. Thus, initially the virus production rate has to be zero. This has been modeled previously by introducing a delay from the time of infection until viral production (Mittler *et al.*, 1998; Nelson *et al.*, 2000; Nelson and Perelson, 2002). Here the optimization calculation may need to be extended in a similar way. In the absence of this extension, we hypothesize that the optimal strategy is one where production starts at zero and then ramps up to the computed fixed optimal rate P^* as rapidly as the biological constraints allow. Such constraints could be incorporated explicitly using a mechanistic model of the within-cell infection process.

If the mortality rate of an uninfected cell is not constant, the optimal schedule for production will vary according to the age of the cell at infection. We assume that the virus has no knowledge of this age (if it could, our previous arguments would hold, but this degree of sophistication seems unlikely). The optimization of the production schedule must therefore be considered over the probability distribution of host cell ages, a , at the time of infection. Specifically, the mean burst size is given by

$$N_a[P(t)] = \int_0^\infty \sigma(a) \int_a^\infty P(t)\sigma(t) dt da \quad (30)$$

where $\sigma(t)$ is the probability of survivorship to time t [equation (1)] and the lower limit of the inner integral is a , since there is no production before infection. $P(t)$ must now be chosen to optimize N_a , which is a lengthier task than that considered in this paper.

Although we have focused on viral infection in this paper and were motivated by modeling HIV infection, the general framework extends to any host-parasite system where the parasite must choose a reproduction rate as a function of time. The concept of background mortality rate which we have used in our description of cells may need to be replaced by more a complex function to describe death of a higher organism. In particular, explicit time- and age-dependence (for example due to seasonal changes or life stage of the host) may need to be considered. However, more complex parasites than viruses will presumably have more flexibility in optimizing their behavior given this kind of complexity in μ .

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APPENDIX A: OPTIMIZATION OF THE BURST SIZE

We wish to find the optimal function $P(t)$ that maximizes

$$\hat{N}[P] = \int_0^{\infty} P(T)e^{-\int_0^T \mu(P(\tau), \tau) d\tau} dT. \quad (\text{A.1})$$

We do this by finding $\delta\hat{N}/\delta P$, the functional derivative of $\hat{N}[P]$ with respect to $P(t)$. Letting $P(t) \rightarrow P(t) + \delta P(t)$, with a corresponding change in burst size, $\hat{N} \rightarrow \hat{N} + \delta\hat{N}$, we find after a little work that the derivative at time t is given by

$$\frac{\delta\hat{N}}{\delta P(t)} = e^{-\int_0^t \mu(P(\tau), \tau) d\tau} - \mu'(P(t), t) \int_t^{\infty} P(T)e^{-\int_0^T \mu(P(\tau), \tau) d\tau} dT \quad (\text{A.2})$$

$$= \sigma(t) \left(1 - \mu'(P(t), t) \int_t^{\infty} P(T)e^{-\int_t^T \mu(P(\tau), \tau) d\tau} dT \right), \quad (\text{A.3})$$

where we have written the survivorship [equation (1)] up to time t as $\sigma(t)$, and we define $\mu' = \partial\mu/\partial P$. This is an integral equation for $P(t)$, and is easily interpreted as follows. First, note that $\sigma(t) > 0$ for all t , so the sign of the derivative is controlled by the parenthetical terms. The first of these comes from differentiating $P(t)$ with respect to itself, and represents the direct increase in \hat{N} due a change in P . The second term represents the loss of future contributions to \hat{N} due to increase in P at the present time.

We also note that because the parenthetical terms are dependent only on future time points (we are integrating from t onwards), the optimal strategy is independent of any earlier production. This type of behavior is predicted for dynamic optimization problems by Bellman's principle of optimality (Bellman, 1957).

Continuing, if $P(t)$ is part of the optimal production schedule then $\delta N/\delta P(t) = 0$, which implies that

$$\int_t^{\infty} P(T) \exp \left[- \int_t^T \mu(P(\tau), \tau) d\tau \right] dT = \frac{1}{\mu'(P(t), t)}. \quad (\text{A.4})$$

If we now differentiate equation (A.3) with respect to t and use equation (A.4) when $\delta\hat{N}/\delta P = 0$ we get the following ordinary differential equation:

$$\frac{dP}{dt} = \frac{1}{\mu''(P(t), t)} \left(\mu'(P(t), t)^2 \left(P(t) - \frac{\mu(P(t), t)}{\mu'(P(t), t)} \right) - \frac{\partial^2 \mu}{\partial P \partial t} \right) \quad (\text{A.5})$$

where $\mu''(P(t), t) = \partial^2 \mu / \partial P^2$. We have not defined any initial or boundary conditions for $P(t)$. Therefore, [equation \(A.5\)](#) defines a one-parameter family of potential optimal solutions, depending on the value of the initial condition $P(0)$. The natural boundary conditions are best written in terms of the survivorship: $\sigma(0) = 1$ and $\sigma(\infty) = 0$. In fact, we can restate the whole problem in terms of σ , as follows. Assuming $\mu(P(t), t)$ is strictly an increasing function of P for all t , we introduce the time-dependent inverse $M \equiv \mu^{-1}$ as follows:

$$-\frac{d}{dt} \ln(\sigma(t)) = \mu(P(t), t) \quad (\text{A.6})$$

$$M \left(-\frac{\sigma'(T)}{\sigma(T)} \right) \equiv \mu^{-1} \left(-\frac{\sigma'(t)}{\sigma(t)} \right) = P(t). \quad (\text{A.7})$$

Now \hat{N} is defined in terms of σ :

$$N[\sigma] = \int_0^L M \left(-\frac{\sigma'(T)}{\sigma(T)} \right) \sigma(T) dT, \quad (\text{A.8})$$

and finding the functional derivative is relatively simple:

$$\frac{\delta \hat{N}}{\delta \sigma} = M \left(\frac{-\sigma'}{\sigma} \right) - \frac{\sigma'}{\sigma} M' \left(\frac{-\sigma'}{\sigma} \right) + \frac{d}{dt} \left\{ M' \left(\frac{-\sigma'}{\sigma} \right) \right\}. \quad (\text{A.9})$$

Setting $\delta \hat{N} / \delta \sigma = 0$ and using the identities [\(A.6\)](#) and [\(A.7\)](#), [equation \(A.9\)](#) becomes

$$\frac{dP}{dt} = \frac{1}{\mu''(P(t), t)} \left(\mu'(P(t), t)^2 \left(P(t) - \frac{\mu(P(t), t)}{\mu'(P(t), t)} \right) - \frac{\partial^2 \mu}{\partial P \partial t} \right) \quad (\text{A.10})$$

as above [\(A.5\)](#). The boundary condition arising from integrating by parts is

$$\left[\frac{\delta \sigma}{\mu'(P(t))} \right]_0^\infty = 0. \quad (\text{A.11})$$

Imposing $\delta \sigma(0) = \delta \sigma(\infty) = 0$ is consistent with the boundary conditions described above, and satisfies this condition. Along with the conditions, [equation \(A.9\)](#) could in principle be used to determine the optimal $\sigma(t)$ and thus the optimal $P(t)$. But practically speaking, it is generally simpler to consider all possible trajectories of [\(A.5\)](#) and choose the best one.

The next step is to identify the criteria for solutions of [equation \(A.5\)](#) which maximize \hat{N} . In order for [equation \(A.5\)](#) to describe the optimal virus production schedule [i.e. the function $P(t)$ which maximizes \hat{N}], $\frac{\delta^2 \hat{N}}{\delta P(t) \delta P(t')}$ must be negative for all t . To examine this, we look at the second functional derivative, and find

$$\frac{\delta^2 \hat{N}}{\delta P(t) \delta P(t')} = -\delta(t - t') \sigma(t) \mu''(P(t), t) \int_t^\infty P(T) e^{-\int_t^T \mu(P(\tau), \tau) d\tau} dT. \quad (\text{A.12})$$

Evaluating this equation at $\delta \hat{N} / \delta P = 0$, and using [equation \(A.4\)](#)

$$\frac{\delta^2 \hat{N}}{\delta P(t) \delta P(t')} = -\sigma(t) \frac{\mu''(P(t), t)}{\mu'(P(t), t)} \delta(t - t'). \quad (\text{A.13})$$

As discussed earlier, $\sigma(t) > 0$, and because we have also assumed that $\mu(P(t), t)$ is a strictly increasing function of P , by definition $\mu'(P(t), t) > 0$. Therefore, the optimality criterion simply reduces to

$$\mu''(P(t), t) > 0. \quad (\text{A.14})$$

[Equation \(A.14\)](#) implies that any solution to [equation \(A.5\)](#) which maximizes \hat{N} will always lie within the realm in which μ is an accelerating function of P . Conversely, a production schedule which minimizes \hat{N} is one which always lies within the realm in which μ is a decelerating function of P .

APPENDIX B: EXPLICIT TIME DEPENDENCE OF THE MORTALITY RATE AND VIRAL PRODUCTION SCHEDULE

Here we examine the general case where both P and μ are functions of t . We will take μ as an increasing, concave up function of P , and further assume that $\partial \mu / \partial t > 0$. From [equation \(A.5\)](#), extremal $P(t)$ evolve in time according to

$$\frac{dP}{dt} = \frac{1}{\mu''(P(t), t)} \left(\mu'(P(t), t)^2 \left(P(t) - \frac{\mu(P(t), t)}{\mu'(P(t), t)} \right) - \frac{\partial^2 \mu}{\partial P \partial t} \right), \quad (\text{B.1})$$

(where $'$ indicates differentiation with respect to P) which can now be non autonomous. Although it is possible that $\mu(P, t)$ depends on interactions of viral production and time, we shall only examine a particular class of functions where the time- and production-dependent parts contribute separately: let $\mu(P(t), t) = f(P) + m(t)$. Then the burst size is given by

$$\hat{N}[P] = \int_0^\infty \sigma_1(t) P(t) e^{-\int_0^t f(P(a)) da} dt. \quad (\text{B.2})$$

The dynamical equation for extremal P (B.1) becomes

$$\frac{dP}{dt} = \frac{f'(P)^2}{f''(P)} \left(P - \frac{f(P) + m(t)}{f'(P)} \right). \tag{B.3}$$

We now investigate a special case of this where an explicit solution can be found: let $\mu(P(t), t) = (\lambda/2)(P/P_{\max})^2 + m(t)$. We believe the behavior of this example to be generic for solutions of (B.3) with concave-up $f(P)$ and increasing $m(t)$.

Rescaling as in Example 2 ($p = P/P_{\max}$, $\tau = \lambda t$, $\phi(\tau) = m(\tau)/\lambda$), the dynamical equation becomes

$$\frac{dp}{d\tau} = p \left(\frac{p^2}{2} - \phi(\tau) \right). \tag{B.4}$$

This can be integrated with initial condition $p(0)$ to get

$$p(\tau) = e^{-\int_0^\tau \phi(a) da} \left(\frac{1}{p(0)^2} - \int_0^\tau e^{-2\int_0^T \phi(a) da} dT \right)^{-1/2}. \tag{B.5}$$

Finite time blow-up of $p(\tau)$ occurs if $p(0) > p^f$ where

$$p^f = \left(\int_0^\infty e^{-2\int_0^T \phi(a) da} dT \right)^{-1/2}. \tag{B.6}$$

Define $\Pi(\tau)$ as the solution of $(p^2/2) - \phi(\tau) = 0$, i.e., $\Pi(\tau) = \sqrt{2\phi(\tau)}$. We now examine four cases:

CASE 1. $p(0) \leq \Pi(0)$.

From equation (B.4), and the fact that $\phi(\tau)$ is increasing, it is clear that $dp/d\tau < 0$ for all τ . $p(\tau)$ will be a monotonically decreasing function, asymptoting to 0 as $\tau \rightarrow \infty$.

CASE 2. $\Pi(0) < p(0) < p^f$.

Note that $\Pi(0) < p^f$ follows from the monotonicity of ϕ . It is clear from (B.4) that if $p(0) > \Pi(0)$ then $dp/d\tau|_{\tau=0} > 0$. Therefore, in this case $p(\tau)$ always increases at first. We now show that there will be a time after which $p(\tau)$ is strictly decreasing. First, we define

$$b(\tau) = (1/p(0)^2) - \int_0^\tau \exp\left(-2 \int_0^T \phi(a) da\right) dT. \tag{B.7}$$

Using equation (B.5),

$$\frac{p^2}{2} = \left(\frac{1}{2} e^{-2\int_0^\tau \phi(a) da} \right) / \left(\frac{1}{p(0)^2} - \int_0^\tau e^{-2\int_0^T \phi(a) da} dT \right) \tag{B.8}$$

$$= \frac{-b'(\tau)}{b(\tau)}. \tag{B.9}$$

$-b'(\tau) \rightarrow 0$ as $\tau \rightarrow \infty$, because $\phi(\tau)$ is an increasing function. In this case, $b(\tau)$ is bounded below as $\tau \rightarrow \infty$. Therefore, for large τ , $(p^2/2) < \phi(\tau)$ and so $dp/d\tau < 0$ [by equation (B.4)]. Hence, $\lim_{\tau \rightarrow \infty} p(\tau) \rightarrow 0$. This case is interesting in that $p(0)$ exceeds the static optimum, but $\phi(\tau)$ increases sufficiently rapidly that it ‘overtakes’ $(1/2)p^2$, blow-up does not occur, and $p(\tau)$ ends up falling to 0.

CASE 3. $p^f < p(0)$.

In this case, $p(\tau)$ blows up in finite time.

CASE 4. $p(0) = p^f$.

This case is the most interesting. Here, there is no finite time blow up of the solution, but $dp/d\tau$ remains positive for all time. We also see that, at long times, $p(\tau) \simeq \Pi(\tau)$, for as $\phi(\tau)$ gets very large, the term $(1/2)p^2 - \phi(\tau)$ from equation (B.4) must remain under control. Further, we speculate that this solution is optimal:

CONJECTURE 2. Let $\mu(p(\tau), \tau) = (1/2)p^2 + \phi(\tau)$, with $\phi'(\tau) > 0$. Then the optimal production rate $p^*(\tau)$ is given by (B.5) with initial condition $p(0) = p^f$ defined by (B.6). Also, $\lim_{\tau \rightarrow \infty} (p^*(\tau) - \sqrt{2\phi(\tau)}) = 0$.

We believe that this can be generalized as follows.

CONJECTURE 3. Let $\mu(P, t) = f(P) + m(t)$ with $f'(P) > 0$, $f''(P) > a > 0$ for some a and $m'(t) > 0$. Then there exists a set of initial conditions $P^f < P(0) < \infty$ for which the solutions of (B.1) undergo finite time blow up. The optimal solution $P^*(t)$ has initial condition P^f , and satisfies

$$\lim_{t \rightarrow \infty} P^*(t) - \frac{\mu(P^*, t)}{\mu'(P^*, t)} = 0. \tag{B.10}$$

The preceding ideas are illustrated using the simplest possible system of this type in the following example.

EXAMPLE 3. Let $\mu(P(t), t) = (\lambda/2)(P(t)/P_{\max})^2 + \delta t + m$. Rescaling as before (Example 2), $\hat{\mu}(p, \tau) = (1/2)p^2 + \epsilon\tau + \phi$ and the general solution from equation (B.5), for extremal p is

$$p(\tau) = e^{-\tau(\epsilon\tau + 2\phi)/2} \left(\frac{1}{p(0)^2} - \frac{e^{\phi^2/\epsilon}}{\epsilon} \left(\int_{\phi}^{\phi + \epsilon\tau} e^{-a^2/\epsilon} da \right) \right)^{-1/2} \tag{B.11}$$

which can also be written in terms of the error function. This solution approaches that of Example 2 for small τ as $\epsilon \rightarrow 0$. The condition for finite time blow up is [from (B.6)]

$$p(0) > p^f = \left(\frac{e^{\phi^2/\epsilon}}{2} \sqrt{\frac{\pi}{\epsilon}} \operatorname{erfc} \left(\frac{\phi}{\sqrt{\epsilon}} \right) \right)^{-1/2}. \tag{B.12}$$

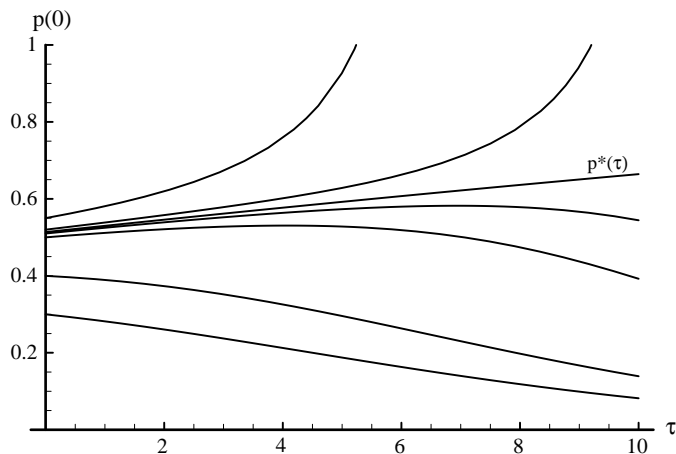


Figure B.1. (Example 3) Solutions of equation (B.5) for different $p(0)$, given $\hat{\mu}(p(\tau), \tau) = (1/2)p^2 + \epsilon\tau + \phi$ and using $\epsilon = 0.01$ and $\phi = 0.1$. Three regimes are visible: (i) finite time blow-up, for $p(0)$ greater than the solution of (B.12), which is $\simeq 0.5137$ for these parameters; (ii) increasing and decreasing solutions for $0.5137 > p(0) > \sqrt{2\phi} \simeq 0.44$; (iii) monotonically decreasing solutions for $p(0) < \sqrt{2\phi}$. The optimal solution p^* satisfies $p^*(0) \simeq 0.5137$.

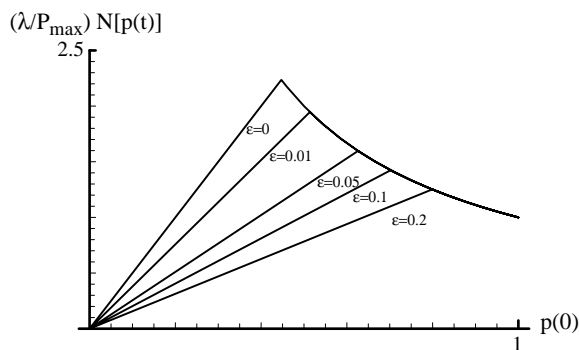


Figure B.2. (Example 3) Burst size as a function of initial condition, determined numerically using $\phi = 0.1$ and $\epsilon = 0, 0.01, 0.05, 0.1, 0.2$, for $\hat{\mu}(p(\tau), \tau) = (1/2)p^2 + \epsilon\tau + \phi$. The optimal burst size for $\epsilon = 0.01$ is found at $p(0) \simeq 0.5137$, just before the onset of finite time blow-up.

Figure B.1 shows a family of extremal $p(\tau)$ for this problem with different initial conditions. The burst size can't be explicitly integrated in this case, so we present a numerically determined plot of $(\lambda/P_{\max})\hat{N}[p]$ against $p(0)$ (Fig. B.2). The optimal initial condition is that corresponding to equality in equation (B.12), in agreement with the first conjecture above. In the case $\epsilon = 0.01$, this turns out to be $p \simeq 0.5137$. We can compare this to the static strategy found in Example 2, which for $\epsilon = 0.01$ was $p \simeq 0.563$. The optimal static schedule is higher than the dynamic optimum at first, because it cannot increase later when survivorship diminishes.

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